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**TITLE: Modeling Marrow Failure and MDS for Novel Therapeutics**

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**RECIPIENT: Children's Hospital Corporation  
Boston, MA 02115**

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14. ABSTRACT Clonal evolution is a potentially life threatening long-term complication of inherited and acquired bone marrow failure (BMF) disorders. Cytogenetic clonal abnormalities develop in approximately 10% of patients with acquired aplastic anemia. The risk of early progression to myelodysplastic syndrome (MDS) and leukemia is also markedly elevated in patients with inherited marrow failure syndromes compared to age-matched controls. Prognosis of different clonal abnormalities is variable. Somatic acquired clonal deletions of one copy of chromosome 7 (monosomy 7) or part of the long arm of chromosome 7 (del7q) are generally associated with a poor prognosis. Deletions involving chromosome 7 are frequently associated with therapy-related MDS, hypocellular MDS, MDS arising in children, and in MDS arising in patients with marrow failure. The goal of this project is to model monosomy 7 arising in marrow failure by utilizing induced pluripotent stem cells (iPSC) derived from patients with the inherited marrow failure/leukemia predisposition syndrome Shwachman-Diamond syndrome (SDS) into which a deletion of the MDS-associated region of 7q has been genomically engineered. We will perform functional genomic screens to identify genes and molecular pathways with synthetic lethality for the del7q clone. These studies will provide a platform for the development of new strategies to treat monosomy 7 clonal disease arising from marrow failure. In light of recent reports describing methodologies to expand iPSC-derived CD34+ cell populations, during the project period, we investigated the possibility of conducting the screen using hematopoietic CD34+ cells. succeeded in expanding CD34+ cells from the SDS iPSC; however, the iPSC carrying the deletion of chromosome 7q failed to expand in sufficient quantities required for screening. Although the effort spent working out the CD34+ expansion required some additional time, we are still on track to complete the aims. We are proceeding with the screen using undifferentiated SDS iPSC+/-del7q. We have validated the feasibility of expanding the del7q clones for high throughput genomic screening and are in the process of conducting the screen using Cas9 with a guide RNA library as outlined in the proposal.					
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**PI: Shimamura, Akiko**

**Title: *Modeling Marrow Failure and MDS for Novel Therapeutics***

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## **1. Introduction**

Clonal evolution is a potentially life threatening long-term complication of inherited and acquired bone marrow failure (BMF) disorders. Cytogenetic clonal abnormalities develop in approximately 10% of patients with acquired aplastic anemia. The risk of early progression to myelodysplastic syndrome (MDS) and leukemia is also markedly elevated in patients with inherited marrow failure syndromes compared to age-matched controls. Prognosis of different clonal abnormalities is variable. Somatically acquired clonal deletions of one copy of chromosome 7 (monosomy 7) or part of the long arm of chromosome 7 (del7q) are generally associated with a poor prognosis. Deletions involving chromosome 7 are frequently associated with therapy-related MDS, hypocellular MDS, MDS arising in children, and in MDS arising in patients with marrow failure. The goal of this project is to model monosomy 7 arising in marrow failure by utilizing induced pluripotent stem cells (iPSC) derived from patients with the inherited marrow failure/leukemia predisposition syndrome Shwachman-Diamond syndrome (SDS) into which a deletion of the MDS-associated region of 7q has been genomically engineered. We will perform functional genomic screens to identify genes and molecular pathways with synthetic lethality for the del7q clone. These studies will provide a platform for the development of new strategies to treat or prevent monosomy 7 clonal disease arising from marrow failure.

## **2. Keywords**

Bone marrow failure, clonal evolution, induced pluripotent stem cells, genomic engineering

## **3. Accomplishments**

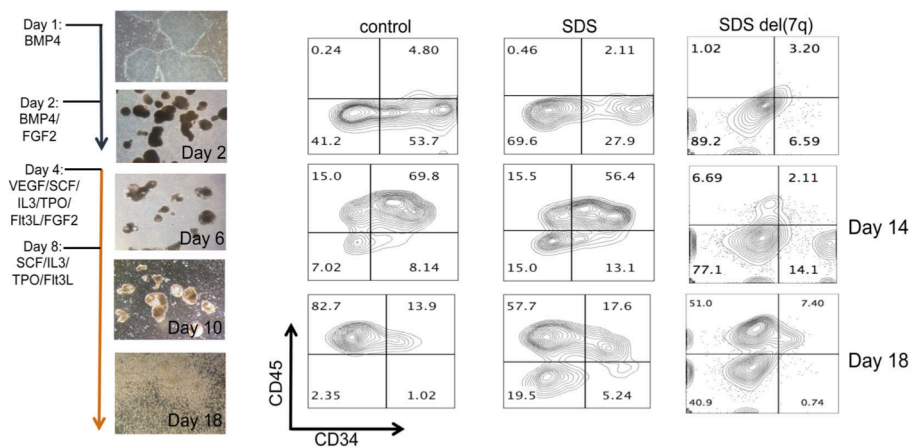
### ***What were the major goals and objectives of the project?***

Aim 1: Conduct a functional genomic synthetic lethality screen targeting del7q clones. (Proposed Timeline: Months 1-12)

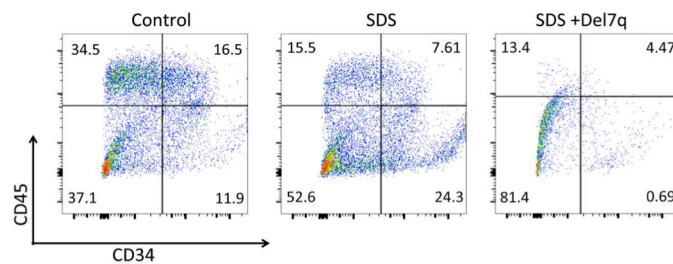
Aim 2: Functional validation of siRNAs selectively lethal for del7q clones (Proposed Timeline: Months 12-24)

### ***What was accomplished under these goals?***

In discussion with Dr. George Daley, a stem cell expert at Harvard, we became aware of new strategies available to expand iPS-derived CD34+ hematopoietic stem/progenitor cells. Conducting the screen in a hematopoietic model would be highly relevant for the study of marrow failure and clonal evolution. This did not change the major goals and objectives of the study. Therefore, we worked on adapting these techniques to the SBDS+/-del7q models. Unfortunately, the CD34+ cell population from the del7q clone failed to expand well, despite successful expansion of the CD34+ population derived from the wild type and SDS lines. (Figure 1) This low yield of a homogeneous del7q CD34+ population (Figure 2) precluded this approach for a high throughput screen.



**Figure 1. Deletion of 7q results in hematopoietic impairment of SDS iPSC.**



**Figure 2: Deletion of 7q results in limited expansion of CD34<sup>+</sup> progenitors.** iPSC-derived CD34<sup>+</sup> hematopoietic progenitors after induced expression of *SOX4*, *MYB*, *HOXA9*, *ERG* and *RORA*.

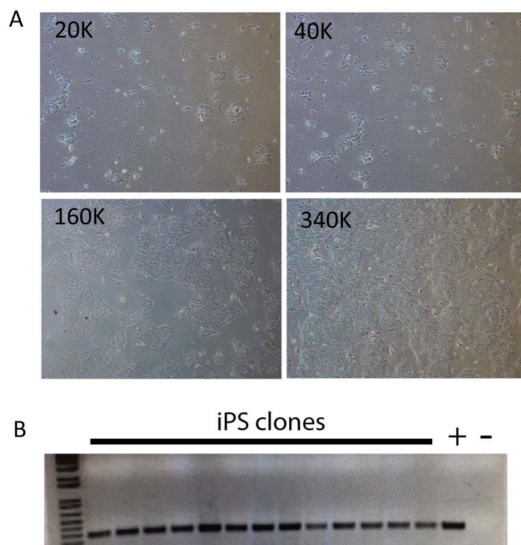
For this reason, the wild type and SDS+/- del(7q) iPSCs are being used in the pluripotent state for the screen. Results will be validated in a hematopoietic model. An additional barrier was the propensity of the del7q line to spontaneously revert to gain an extra copy of chromosome 7. (Figure 3) Careful attention to low passage and regular karyotyping has been required, which has delayed the anticipated pace of the project.



**Figure 3: Reversion of SDS del(7q) clones.** Karyotype of SDS del(7q) clone showing spontaneous reversion by duplication of chromosome 7. Karyotype: 47,XX,+del(7q).

Cas9 has been stably introduced into the iPSC lines without apparent adverse effects and measurement of expression levels is ongoing in preparation for the screen. (Figure 4)

Given the very slow growth and poor hematopoietic differentiation of the del7q line, we are also testing whether knockdown of p53 will rescue the hematopoietic defect in the SDS del7q line. P53 mutations are common in MDS arising in SDS patients, so this approach is clinically relevant.



**FIGURE 4: Stable integration of CAS9-expressing plasmid does not impair SDS iPSC.** A) SDS iPSC were transduced with a lentivirus expressing a blastocidin-resistance gene and CAS9. Clones were selected with blastocidin. Morphology and optimal plating density of a CAS9-expressing clone is shown. (B) PCR for CAS9 in blastocidin-resistant clones. + blastocidin vector; - water control

***What opportunities for training and professional development did the project provide?***

This project has provided opportunities for Dr. Melisa Ruiz--Gutierrez, a female Hispanic trainee, to train in the field of bone marrow failure. This project will provide a platform for her future career. This project also supports a postdoctoral PhD fellow, Dr. Ozge Bolukbasi who is working on this project with Dr. Ruiz-Gutierrez. Dr. Ruiz-Gutierrez presented her work on the SBDS+/- del7q system at the American Society of Hematology meeting and the K12 Hematology Training Grant meeting.

***How were the results disseminated to communities of interest?***

Preliminary data was presented at the American Society of Hematology and at the K12 Hematology conference.

***What do you plan to do during the next reporting period to accomplish the goals and objectives?***

We will complete the functional genomic screen and investigate high priority candidate genes using the approaches outlined in the proposal.

**4. Impact**

**What was the impact on the development of the principal discipline(s) of the project?**

Monosomy 7/del7q MDS is associated with a high risk of progression to leukemia and carries a poor prognosis. Chromosome 7 losses are frequently noted in MDS arising in patients with inherited or acquired marrow failure. The only curative treatment currently available for monosomy 7/del7q MDS is a hematopoietic stem cell transplant, but success is limited by donor availability, treatment-related toxicities, graft-versus-host disease, and relapse. The paucity of disease models for monosomy 7/del7q MDS poses a significant barrier to the development of effective medical therapies. This project builds on a novel iPSC model which will advance our understanding of the molecular and hematologic consequences of del7q arising in the context of marrow failure. This study stands to identify target genes and molecular pathways for the development of new strategies to treat or prevent monosomy 7/del7q clonal evolution arising from marrow failure. Genes and pathways identified in this study will also be interrogated in primary bone marrow samples from MDS patients (with and without monosomy 7/del 7q) as well as in MDS arising in the context of other marrow failure syndromes in future studies. Another barrier to treatment is the lack of sensitive and specific biomarkers identifying marrow failure patients at high risk for MDS and leukemia. These studies may also begin to elucidate potential molecular biomarkers for patients who would benefit from early transplant to prevent progression to high grade MDS and leukemia.

**What was the impact on other disciplines?**

Nothing to report.

**What was the impact on technology transfer?**

Nothing to report.

**What was the impact on society beyond science and technology?**

Project still in progress so nothing to report yet.

## **5. Changes/Problems**

**Changes in approach and reasons for change**

We investigated the possibility of deriving and expanding a CD34+ hematopoietic progenitor population from the SBDS+/-del7q iPSC. Although we were successful with the SBDS iPSC, the del7q line failed to expand sufficiently despite intensive efforts to optimize the differentiation conditions. The slow growth and propensity for reversion of the del7q iPSC has also delayed the pace of the project but we anticipate that the goals of the project can still be completed within the funding period.

**Actual or anticipated problems or delays and actions or plans to resolve them**

Challenges and alternative approaches were discussed in section 3. These challenges have delayed the timeline of this project; however we are also proceeding with targeted screens of candidate pathways. These complementary targeted functional genomic approaches will provide important mechanistic insights into del7q-selective vulnerabilities.

## **Changes that had a significant impact on expenditures**

Nothing to report

## **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Although this project is covered by a larger protocol involving additional studies, at the request of the DoD an IRB protocol specifically focused on just this project was submitted and approved by our institutional IRB. IRB protocol P00024012 for the DoD-specific project was approved on 11/02/2016.

## **6. Products**

Nothing to report.

## **7. Participants & Other Collaborating Organizations**

### ***Participants***

**Akiko Shimamura, MD PhD** is a physician-scientist in pediatric hematology oncology. Her research focuses on bone marrow failure and MDS. Her laboratory has developed assays and reagents to study these disorders. She also has a clinically annotated marrow failure/MDS repository with data and samples from approximately 450 patients and their family members. Dr. Shimamura serves as Principal Investigator on this study and is overseeing experimental design, data analysis, and manuscript preparation.

**Melisa Ruiz-Gutierrez, MD PhD** is a physician scientist postdoctoral fellow in Dr. Shimamura's lab. Her background training spans molecular biology, biochemistry, cell biology, and induced pluripotent stem cell biology. She analyzed the data for the siRNA screen and is performing the functional validation assays together with Ozge Bolukbasi. No salary support is requested since Dr. Ruiz-Gutierrez is supported by a K12 training grant.

**Ozge Bolukbasi** is a postdoctoral fellow in the Shimamura lab. She is working with Dr. Ruiz-Gutierrez on the screen and functional assays.

**Kaitlyn Ballotti**. Is the research technician responsible for maintenance of the iPSC cultures. These cells are labor-intensive to culture and require ongoing daily time-consuming work. This technical support is critical for completion of this project in the 2-year time frame.

### ***Collaborating Organizations***

No collaborating organizations to report.

## **8. Special Reporting Requirements**

Not applicable.

## **9. Appendices**

Not applicable.